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Oxygen Response in *Lactococcus lactis* subsp. *lactis* during Milk Fermentation

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The acidification activity (i.e. ΔpH) of primary starter cultures used for cheese production is influenced by the initial oxygen content of milk. Acidification of milk is an important parameter for the final cheese quality. Since prior results have shown that ΔpH increased with oxygen depletion, we wanted to characterise the oxygen response of *Lc. lactis* subsp. *lactis* during fermentation of full fat milk. DNA microarray analysis was used to identify significant differentially expressed genes affected by oxygen depletion. Both milk with initial high (300%) and initial low (30%) oxygen content was investigated.

The preliminary results of this study showed that genes that were down-regulated at oxygen depletion were involved in removal of oxygen and different redox reactions. Genes up-regulated at oxygen depletion were related to anaerobic growth, oligopeptide transport which is also important for growth, and stress responses like acid adaptation.

Study of the affected genes will provide knowledge about how the properties of the starter culture are influenced by the oxygen conditions. This knowledge can help the dairy industry to decide whether to change the initial oxygen content of the milk or to select new starter cultures with higher oxygen tolerance in order to save time on the acidification process.

Keywords: Acidification activity, Oxygen Sensitivity, Redox Potential, Gene Expression



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Introduction

The acidification activity of primary starter cultures used for cheese production is influenced by the initial oxygen content of milk. Acidification of milk is an important parameter for the final cheese quality. Knowledge about how a starter culture responds to the changing oxygen conditions during milk fermentation and cheese production will be useful to the dairy industry. It will support the development of technological procedures and new starter cultures.

Objective

In this preliminary study we wanted to characterise oxygen response of *Lc. lactis* subsp. *lactis* during fermentation of full fat milk. DNA microarray analysis have been used to identify significant differentially expressed genes affected by oxygen depletion. Both milk with initial high (300%) and initial low (30%) oxygen content have been investigated. The identified genes will be used to describe which biological functions are influenced by oxygen depletion.

Up-regulated genes

Alcohol metabolism (*adhE*)

- Regeneration of NADH
- Mixed acid fermentation in presence of O₂

Peptide transport (*oppF*)

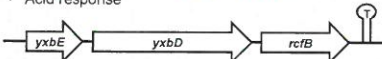
- Growth in milk
- Oligopeptide transport system (*oppDFBCA*)⁵
- Other genes from operon:
Fold-change < 2, but show same profile

Arginine/Ornithine metabolism (*arcABC1C2D1*)

- Acid stress
- Production of intracellular NH₃

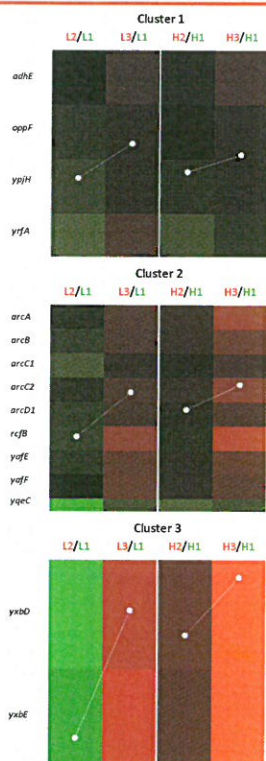
Stress response (*rcfB*, *yxhE*)

- Possibly O₂ response (L2/L1 vs. H2/H1)
- Acid response



Anaerobic growth (*nrdD*, *nrdG* – results not shown)

- Anaerobic ribonucleotide reductase (Class III)
- Provides dNTP's for DNA synthesis⁴
- Upregulated, while the corresponding aerobic ribonucleotide reductase (Class I) is downregulated



Down-regulated genes

Cysteine, methionine and sulfur metabolism (*cysK*, *metB2*)

- Redox related – thiol groups and disulfide bonds
- *cysK* – induced by methionine and superoxide (O₂⁻)

Transport of manganese (*mtsABC*, *pabAB*, *ykJBC*)

- Cofactor for many enzymes
- Required by eg. superoxide dismutase (*sodA*)
- *sodA* – constitutively expressed
2 O₂⁻ + 2 H⁺ → H₂O₂ + O₂ (superoxide dismutase)

Pyrimidine, purine, nucleoside and nucleotide metabolism (*purN*, *carA*, *carB*, *pnuC2*, *pyrRBP*, *pyrC*, *pyrZ*, *dukA*)

- Growth related – de novo synthesis²
- Repair due to oxygen damage

Pyruvate dehydrogenase component (*pdhD*)

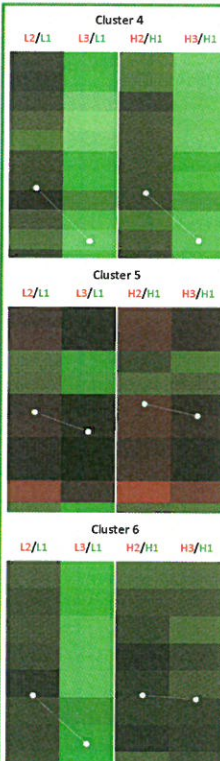
- Regeneration of NADH
- Mixed acid fermentation in presence of O₂
- Other genes from operon (*pdhABCD*):
Fold-change < 2, but show same profile

Oxygen removal, cell redox homeostasis (*noxE*)

- Removes oxygen
- Competes with LDH for NADH → less lactate produced
2 NADH + 2 H⁺ + O₂ → 2 NAD⁺ + 2 H₂O

Ferrichrome (Fe³⁺) transport (*fhuDg*)

- Redox related
- Product of Fenton reaction³:
H₂O₂ + Fe²⁺ + H⁺ → OH⁻ + H₂O + Fe³⁺



Acknowledgement

In-house microarray facility was provided by Chr. Hansen A/S, Hørsholm, Denmark.

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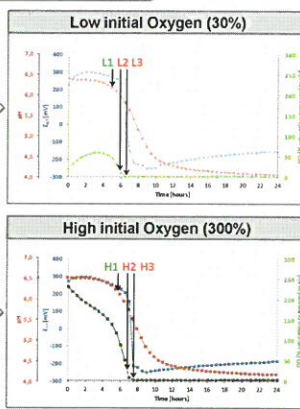
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Materials & Methods

Full fat milk
+ N₂ (g)
+ 10⁸ CFU of
Lc. lactis subsp.
lactis DSM20481



The diagrams show the development of pH, redox potential (Eh) and dissolved oxygen (DO (in % saturation compared to air)) during 24 hours of milk fermentation.

RNA extraction:
L1 + H1, reference at pH 6.2
L2 + H2, before O₂ depletion
L3 + H3, after O₂ depletion
↓
RT to cDNA + postlabelling with
Cy3 (reference)/Cy5 (test)
↓
Hybridisation to oligo-array based on
Lc. lactis subsp. *lactis* IL1403,
printed on an in-house microarray
platform.
Low initial oxygen: L2/L1 & L3/L1
High initial oxygen: H2/H1 & H3/H1
↓
Normalisation: ratio and Lowess
83 genes = Fold-change > 2
66 genes assigned to 12 clusters

Conclusion

Genes up-regulated at oxygen depletion are involved in:

- Growth at anaerobic conditions
- Oligopeptide transport, important for growth in milk
- Stress response
- Acid adaptation through increase of pH by NH₃ production.

Oxygen depletion causes down-regulation of genes related to:

- Removal of oxygen
- Redox reactions

The knowledge obtained in this study may be used to optimise milk processing procedures and cheese starter cultures.

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